REMARKS

The present invention relates to methods and compositions for the differentiation of human preadipocytes isolated from adipose tissue into adipocytes. The invention further relates to methods and compositions for the identification of novel polypeptides secreted from human adipocytes.

Claims 16-31 are currently pending in the application. That is, claims 1-5 were canceled in the Amendment dated November 15, 2001, and claims 6-15 were canceled in the Amendment dated November 14, 2003.

Claim 16 has been amended herein to clarify and to better distinctly claim applicants' invention. Moreover, claim 16 has been amended herein to recite that the preadipocytes are primary cultures, and to recite a fractionating step. Support for the amendment to claim 16 is found throughout the as-filed specification as fully set forth below. Further, claims 17-20 and 29 have been amended to merely clarify that the medium is a differentiation medium, and claim 27 has been amended to depend from claim 16 rather than claim 26. As such, no new matter has been added by way of the these amendments.

In addition, claim 31 has been amended herein as suggested by the Examiner in order to better distinctly claim the subject matter which applicants regard as the invention.

Rejection of claim 16-31 under 35 U.S.C. § 112, first paragraph – new matter

The Examiner has rejected claims 16-31 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time of filing of the present application, had possession of the claimed invention. That is, it is the Examiner's view that the pending claims are broader than the disclosure as set forth in the as-filed specification, and that the current pending claims are broader than the originally filed claims. Specifically, the Examiner contends that the pending claims lack essential steps as disclosed in the specification and the originally filed claims.

As an initial matter, Applicants respectfully contend that the broad interpretation of claim 16 is supported by the as-filed specification. Specifically, support for a method of identifying a protein or a novel polypeptide secreted from a human adipocyte is found beginning on line 27 of page 4. Further, support for a method of identifying a protein or a novel

polypeptide from a human adipocyte comprising isolating a human preadipocyte is found in Example 1, beginning on page 17. Therefore, Applicants assert that there is no requirement for a step comprising fractionating the conditioned media. However, Applicants, while not necessarily agreeing with the Examiner's reasoning, but rather in a good faith effort to expedite prosecution of this application, have amended claim 16 to recite a step comprising "fractionating the conditioned medium of the differentiated adipocytes." The amendment to claim 16 with respect to a fractionation step is fully supported by the specification as filed. For example, beginning on line 25 of page 16, the specification discloses that an embodiment of the present invention encompasses a method for identifying proteins and peptides secreted from cultured human adipocytes, comprising fractionating the conditioned media of adipocytes differentiated from preadipocytes by the methods of the invention. Accordingly, no new matter has been added by way of this amendment.

The Examiner further contends that the specification does not support "comparing" the pattern of proteins, polypeptides or peptides secreted. Applicants assert that the specification fully supports "comparing." For Example, Figure 8 depicts an autoradiograph of human preadipocyte and differentiated adipocyte secreted proteins separated by isoelectric focusing and two-dimensional gel electrophoresis. One skilled in the art would appreciate, based upon the present disclosure and Figure 8, that a further embodiment of the present invention encompasses comparing the pattern of secreted proteins from preadipocytes and adipocytes. Therefore, Applicants respectfully contend that the as-filed specification fully supports a step comprising comparing the pattern of proteins.

Claim 16 as presently amended, recites steps supported by the specification as filed and, therefore, the rejection of claims 16-31 under 35 U.S.C. § 112, first paragraph should be reconsidered and withdrawn.

In addition, the Examiner has also rejected claim 31 under 35 U.S.C. §112, because the Examiner contends that the term "genetically modified" is not supported by the as-filed specification. While not necessarily agreeing with the Examiner's reasoning, but rather in a good faith effort to expedite prosecution of this application, Applicants have amended claim 31 as suggested by the Examiner to indicate that exogenous DNA may be introduced into the cells of the present invention in order to render the cells genetically modified. Support for the amendment to claim 31 relating to introducing exogenous DNA into a cell is found in the as-filed

specification. For example, beginning on line 26 of page 15, various methods such as, but not limited to, calcium phosphate, lipofectin and viral infection are disclosed for introducing exogenous DNA into the cells.

Applicants respectfully request reconsideration and withdrawal of the Examiner's rejection of claim 31 under 35 U.S.C. §112 in view of the amendment to claim 31.

Rejection of claims 16-31 pursuant to 35 U.S.C. §103(a)

The Examiner has rejected claims 16-31 under 35 U.S.C. §103(a) as being *prima facie* obvious over Zilberfarb et al. (1997, J. of Cell Science 110:801-807; "Zilberfarb") or Strosberg et al. (U.S. Pat. No. 6,071,747; "'747") in view of the Gibco Catalog. Specifically, the Examiner opines that Zilberfarb discloses a method of isolating a protein from preadipocytes, and that Strosberg discloses a method of studying proteins expressed by adipocytes differentiated from preadipocytes. Further, the Examiner asserts that the Gibco Catalog discloses various media, for example DMEM/F12 which contains 3.15 g/L glucose. Therefore, the Examiner reasons that it would have been *prima facie* obvious for one skilled in the art to combine the teachings of these references to arrive at the present invention as recited in claims 16-31. Applicants respectfully traverse the rejection.

The three-prong test which must be met for a reference or a combination of references to establish a *prima facie* case of obviousness has not been satisfied in the instant matter. The MPEP states, in relevant part:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). MPEP § 2142

None of these criteria have been met here.

The first prong of the *In re Vaeck* test, the requirement that the references themselves or the knowledge in the art must provide some suggestion or motivation, has not been met in this instance. The Examiner states that Zilberfarb teaches a method of isolating a protein

from preadipocytes (PAZ-6 cells) which have been differentiated into adipocytes, and '747 discloses a method of studying proteins expressed by adipocytes differentiated from preadipocytes, but admits that both primary references do not teach comparing the pattern of proteins secreted by the differentiated cell versus the preadipocyte. The Examiner, however, states that one of ordinary skill in the art would have been motivated to arrive at the present invention because both Zilberfarb and '747 teach that PAZ-6 cells produce leptin once the cells have been converted into adipocytes and may be a model for studying proteins expressed by the adipocytes.

Applicants point out that both Zilberfarb and '747 disclose the use of PAZ-6 cells which are immortalized cells that have been passaged for several months. Specifically, PAZ-6 cells are transfected with the SV40 large and small T-antigens under the control of the vimentin promoter. One skilled in the art would recognize that PAZ-6 cells are immortalized based on the introduction of the SV40 antigens and, therefore, PAZ-6 cells are not primary cultured cells as recited in the presently amended claims. Following entry of the present Amendment, the claims relate to a method of identifying a protein, a polypeptide or a peptide secreted from a human adipocyte derived from a primary culture of a human preadipocyte and then differentiating the human preadipocytes with a differentiation medium. Support for the use of primary cultures of preadipocytes can be found throughout the specification. For example, page 4, lines 13-16 discloses that the present invention provides methods and compositions for the consistent and quantitative differentiation of human preadipocytes isolated from adipose tissue into adipocytes bearing biochemical, genetic and physiological characteristics similar to that observed in isolated primary adipocytes. Further, "preadipocyte" is defined in the specification to refer to cells that could be isolated from a stromal vascular fraction prepared from adipose tissue, and that have the potential to differentiate into adipocytes. In addition, beginning on line 25 of page 9, the specification discloses that human adipose tissue from a variety of sources may be processed to produce preadipocytes for the generation of adipocytes. The as-filed specification does not indicate that the cells of the present invention are immortalized. Thus, Applicants respectfully contend that the cells of the present invention are cells isolated from human tissue which have characteristics of primary cultured cells and are not cells which have been immortalized in cell culture. Again, nowhere does Zilberfarb or '747 teach or suggest using a primary culture cell to identify a protein. Rather, these references teach using an immortalized cell line.

In addition to the lack of the primary references to teach Applicants' cell, the Examiner has also pointed out that neither reference teaches comparing the patterns of proteins secreted by the differentiated cell versus the preadipocyte. Therefore, neither does Zilberfarb or '747 teach or suggest an important feature of the present invention. Certainly, the disclosure of Zilberfarb or '747 does not provide any guidance as to how one skilled in the art might arrive at the present invention as set forth in the pending claims. A mere hint that PAZ-6 cells (preadipocytes) produce leptin once they have been differentiated into adipocytes is not enabling and cannot be considered a "teaching or suggestion" for using primary cultures of preadipocytes and differentiating the primary cultures of preadipocytes into adipocytes to identifying a protein, a polypeptide or a peptide secreted from a adipocyte, as set forth in the presently amended claims.

The Examiner has cited the Gibco Catalog to demonstrate various culture media, specifically DMEM/F12 containing 3.15 g/L glucose. The Gibco Catalog does not correct the deficiencies of either Zilberfarb or '747. In essence, these references (1) do not teach or suggest a using primary cultured preadipocytes and differentiating the primary cultured preadipocytes into adipocytes to identifying a protein, a polypeptide or a peptide secreted from a adipocyte and (2) do not motivate one of skill in the art to combine any them to arrive at the present invention.

The second prong of the *In re Vaeck* test, the requirement that there be a reasonable expectation of success, is similarly not met in this instance. As detailed above, Zilberfarb and '747 merely disclose an immortalized cell line, and do not teach or suggest using primary cultured preadipocytes and differentiating the primary cultured preadipocytes into adipocytes to identifying a protein, a polypeptide or a peptide secreted from a adipocyte as recited in the pending claims. One skilled in the art would understand that the disclosure set forth in Zilberfarb or '747, specifically an immortalized cell line, cannot be used to arrive at Applicants' invention. That is, without further information, which Zilberfarb and '747 do not provide, one of skill in the art has no expectation of success in using the cells of the present invention. If the skilled artisan were to follow the teachings of either Zilberfarb or '747, the skilled artisan would not arrive at the present invention because neither Zilberfarb or '747 teach using primary cell cultures. Therefore, there would have no expectation of success to arrive at Applicants invention based on the teachings of Zilberfarb or '747. As noted above, the Gibco Catalog does not overcome the deficiencies in both Zilberfarb and '747 to teach or suggest

Applicants' cells. That is, none of these references teach using primary cultures of preadipocytes and differentiating the primary cultures of preadipocytes into adipocytes to identifying a protein, a polypeptide or a peptide secreted from a differentiated adipocyte. Therefore, the skilled artisan has no reasonable expectation of success in arriving at the present invention.

In addition to the requirements set forth above, in order to establish a *prima facie* case of obviousness, the prior art reference(s) must teach or suggest all of the claim limitations. Similar to the other prongs of the *In re Vaeck* test, Zilberfarb or '747 in view of the Gibco Catalog fails to teach or suggest all of the claim limitations. Applicants respectfully contend that as discussed elsewhere herein, the cited references, whether alone or in combination, do not teach all of the claim limitations. Zilberfarb and '747 do not teach Applicants' cell or the use thereof to identify proteins. Further, the Gibco Catalog also does not teach the invention as discussed in detail elsewhere herein. Each of these references is fatally deficient with respect to the present invention and none are capable of correcting the deficiencies of the others. The three prong test to establish a *prima facie* case of obviousness has not been met and Applicants, therefore, request withdrawal of the Examiner's rejection of the claims under 35 U.S.C. §103(a).

Summary

Applicants respectfully submit that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that each of currently pending claims 16-31 is in condition for allowance. Reconsideration and allowance of claims 16-31 are respectfully requested at the earliest possible date.

Respectfully submitted,

HALVORSEN ET AL.

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(Date)

By: _____

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Enclosure: Petition for a two-month extension of time and fee therefor